petition the Commissioner for a 1-month extension of time: a separate petition accompanies this amendment.

I. Amendments

Claims 8 and 13 have been amended to re-introduce the size limitation that was deleted in the first preliminary amendment. Hence, support for the size limitation is in claims 1 and 13 as originally filed, and also in the specification, for example, on page 23, lines 11-19. The claims have also been amended to recite that the liposomes include between 1-20 mole percent of the polymer-derivatized lipid, as set forth on page 21, line 27.

Accordingly, the amendments to the claims introduce no new matter.

II. Rejection Under 35 U.S.C. §112, second paragraph

Claims 13-19 were rejected under 35 U.S.C. §112, second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. Specifically, the Examiner asserts that the method step of " entrapping the agent" is indefinite since a method claim should recite how a compound is entrapped. The applicants respectfully traverse this rejection for the following reasons.

A. The 112, Second paragraph Standard

According to the MPEP 2173 and 2173.02, the primary purpose of the requirement that the claims must particularly point out and distinctly claim the invention is to ensure that the scope of the claims is clear so the public is informed of the boundaries of what constitutes infringement of the patent. Definiteness of claim language must be analyzed, not in a vacuum, but in light of

(a) the content of the particular application disclosure;

(b) the teachings of the prior art; and

(c) the claim interpretation that would be given by one possessing the ordinary level of skill in the pertinent art at the time the invention was made.

B. The Rejected Claim 13

Claim 13, in pertinent part, reads:

"A method of preparing a therapeutic agent for localization in an infected region of tissue, when the agent is administered by intraveneous injection, comprising

entrapping the agent in liposomes which:..."

C. Analysis

It is the applicants' position that the metes and bounds of claim 13 are clear to one of skill in the art when read in light of the specification. Claim 13 is concerned with a method of preparing a therapeutic agent for administration by entrapping the agent in a liposome. The application on pages 24, line 29 to page 29, line 4 sets forth several techniques for entrapping a compound in liposomes. For example, the compound can be entrapped via passive incorporation (page 25, line 1 to page 27, line 19) or by active loading (page 27, line 31 to page 29, line 4). These techniques are known and understood by those of skill in the art.

Thus, the step of " entrapping the agent" as set forth in claim 13, is readily understood to mean exactly what is said - the agent is entrapped in the liposomes. In this way, the agent is prepared for administration and localization at an infection site.

The Examiner's request to set forth how the compound is entrapped overlooks the teaching in the specification that a variety of methodologies (passive entrapment, active entrapment) are suitable for entrapping a compound in liposomes. The request also seems misplaced. For example, a claim to " a method of making a composition x, comprising; adding Y..." does not require a description of how Y is added.

Accordingly, applicants respectfully request withdrawal of the rejection under 35 U.S.C. §112, second paragraph.

III. Double-Patenting Rejections

Claims 8-9, 11-12 and 14-17 were rejected under the judicially created doctrine of obviousness-type double patenting at being unpatentable over claims 1-33 of U.S. Patent No. 5,103,556.

Claims 8-19 were provisionally rejected under the judicially created doctrine of obviousness-type double patenting at being unpatentable over claims 1-15 of U.S. Patent No. 5,213,804.

Enclosed herewith is an executed Terminal Disclaimer filed in accordance with C.F.R. §1.321(b) and (c) which disclaims the terminal portion of any patent issuing on the instant application that extends beyond the expiration of U.S. Patent Nos. 5,103,556 and 5,213,804.

The applicants submit that Terminal Disclaimer overcomes the rejection for obviousness-type double patenting and withdrawal of the rejection is respectfully requested.

IV. Rejection under 35 U.S.C. §102

A. Rejection under 35 U.S.C. §102(b)

Claims 8-9 and 17 were rejected under 35 U.S.C. §102(b) as being anticipated by Sears (EP 0 118 316). This rejection is respectfully traversed for the following reasons.

Summaries of the present invention and of the cited Sears reference can be found in the amendment submitted April 12, 1999.

1. The Examiner's Position

It is the Examiner's position that: (1) the Sears reference teaches the amphiphilic compound phosphatidylethanolamine-polyethylene glycol (PE-PEG) for use in encapsulating drugs; and (2) the method of preparation using this lipid is the classical method of liposome preparation (hydration of a dried lipid film with an aqueous medium), and therefore forms the same product as in the instant invention.

2. The Sears Teaching

Sears describes a phospholipid compound where the polar head group of the lipid phosphatidylethanolamine (PE) has been modified to include a polyalkylene oxide polymer, and the polymer polyethylene glycol is exemplary, to give a PE-PEG lipid. Sears describes the lipid on page 8, lines 7-11 as an analog to gangliosides, where the presence of a hydrophilic oligosaccharide on the polar region of the molecule allows the ganglioside to organize into a stable micelle upon hydration.

In Examples 10-14 of the Sears reference, exemplary formulations using the PE-PEG lipid are set forth. In Example 10, Taxol® and the PE-PEG lipid are dissolved in an organic solvent and

then the solvent is removed leaving a thin lipid film. The film is then hydrated with buffer. Examples 11-14 describe the same procedure using different drugs.

In each of the Examples 10-14, the PE-PEG lipid is the sole lipid component. It is known in the art, and as supported by the two enclosed references of Hristova *et al.* [1] and Kenworthy *et al.*[2], 100% of polymer-lipid in water forms micelles. As reported in these references, a mixture of lipid and polymer-lipid forms liposomes only in a certain polymer-lipid concentration range which depends on the nature of the lipid and on the molecular weight of the polymer in the polymer-lipid. For example, for polymers having a molecular weight of about 2000 Da in combination with a saturated lipid (such as DSPC with cholesterol), above about 15 mole percent polymer-lipid results in micelle formation (see highlighted portions of enclosed references).

3. The Applicants' Rebuttal

A. State of the Art

Since the beginning of *in vivo* liposome drug delivery studies in the early 1970's, the major obstacle to their use as drug delivery vehicles was the fact that after administration the liposomes are rapidly removed from the blood by the liver and spleen mononuclear phagocytes. The liposomes fail to remain in the blood long enough to reach a target site or to provide for efficient and specific delivery to any organs other than the spleen and liver (Stealth Liposomes, Lasic and Martin, Eds., CRC Press, (1995), Preface, copy enclosed).

From the early 1970's to mid 1980's, scientists worked on this problem, trying to find a way to circumvent rapid removal of the liposomes by the reticuloendothelical system (RES). The most successful approach was use of small unilamellar vesicles (SUVs) which due to their small size (less than about 70-100 nm) survive in circulation for several hours. However, the small size of the SUVs translates into a small internal volume for carrying drug, making

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[1] Hristova, K. and D. Needham, " Physical Properties of Polymer-Grafted Bilayers" in Stealth Liposomes, CRC Press, (1995) Chapter 5, pages 35-49

[2] Kenworthy, A., *et al.*, *Biophysical Journal*, 64:A348 (1993).

these small vesicles impractical for therapeutic applications.

It was not until 1987 that the beginnings of a solution to the problem of rapid clearance by the RES was reported. In 1987 researches found that liposomes modified with carbohydrate moieties could evade the RES for longer periods of time. A few years later, it was shown that a polymer coating on the liposome can also improve the liposome blood circulation time (Stealth Liposomes, Lasic and Martin, Eds., CRC Press, (1995), Preface, copy enclosed).

B. Sears in view of the State of the Art

The cited Sears reference has a priority date of 1983, well before the earliest recognized 1987 reports on use of carbohydrates to protect liposomes from RES clearance. The Sears reference when taken in view of the state of the art at the time of the Sears invention provides no more than a teaching of a polymer-lipid derivative for use in solubilizing hydrophobic compounds via micelle formation (page 14, lines 12-14 of Sears).

This interpretation of the Sears teaching is consistent with the state of the art in the liposome field, and also with the teaching in the Sears reference itself. Sears states that the polymer-lipid of his invention is essentially a ganglioside analog, and gangliosides organize into stable micelles upon hydration (page 8, lines 5-11). Sears further states that the structures formed using the polymer-lipid of his invention are distinct and unique as compared to liposomes (page 8, lines 31-32). Further, the Examples 10-14 in Sears set forth formulations using the polymer-lipid derivative as the sole component, which as discussed above, can only yield micelles.

Sears is not concerned with forming liposomes or in providing a formulation with a long blood circulation lifetime. Because he is not concerned with the problems address by the present invention, Sears cannot be said to provide a solution.

The invention as claimed is directed to a liposome composition composed of (i) vesicle-forming lipids and (ii) between 1-20 mole percent of a polymer-derivatized lipid. The liposomes have an extended blood circulation lifetime and are able to accumulate selectively in infected tissue.

Nowhere are these claimed features shown in the Sears reference. Thus, Sears cannot be said to anticipate the invention, and withdrawal of the rejection under 35 U.S.C. §102(b).

V. Rejection Under 35 U.S.C. §103

Claims 8-19 were rejected under 35 U.S.C. §103 as being obvious over Sears (EP 0 118 316).

Claims 8-19 were further rejected under 35 U.S.C. §103 as being obvious over Janoff (U.S. Patent No. 4,897,384) or Popescu (U.S. Patent No. 4,981,692) in view of Yoshioka (U.S. Patent No. 5,593,622).

These rejections are respectfully traversed for the following reasons.

Summaries of the present invention and of the cited references can be found in the amendment submitted April 12, 1999.

A. Analysis: Rejection Over Sears

1. The Examiner's Position

The Examiner's position is that Sears teaches lipids of phosphatidylethanolamine derivatized with polyethylene glycol (PE-PEG) and that this PE-PEG lipid is used to encapsulate drugs. The method of encapsulation is via the classic dried lipid film technique. It would be obvious to use this teaching with any drug or any other polymers with the expectation of obtaining similar results.

2. The Applicants Rebuttal

As discussed above, the Sears teaching when taken in light of the state of the art in 1983, provides no more than a teaching of a polymer-lipid derivative for use in solubilizing hydrophobic compounds via micelle formation (page 14, lines 12-14 of Sears). It is clear that micelles are formed using the Sears methodology, since the polymer-lipid is the sole lipid component, and as the references discussed above (Kenworthy *et al.* and Hristova *et al.*) 100% of such a polymer-lipid conjugate can thermodynamically only form micelles.

More importantly, Sears is not concerned with increasing the blood circulation lifetime of liposomes. There is no suggestion to

use the polymer-lipid conjugate in the claimed range of 1-20 mole percent. Nor is there any suggestion in the reference that blood liposome levels could be increased by addition of the claimed polymer moieties to the outer liposome surface.

For these reasons, pending claims 8-19 patentably define over the Sears reference.

B. Analysis: Rejection Over Janoff or Popescu in view of Yoshioka

1. The Examiner's Position

It is the Examiner's position that the attachment of PEG to the surface of liposomes through coupling with the liposome phospholipid (as taught by Yoshioka), would have been an obvious modification to the liposomes of Janoff or Popescu, motivated by Yoshioka's teaching that PEG prevents the adsorption of plasma proteins to the liposome surface and the subsequent agglutination.

The Examiner takes the position, in response to the applicants earlier arguments, that (1) one of skill would be motivated to combine Janoff with Yoshioka to couple PEG based on the teaching of Yoshioka; and (2) there is no data of record to support the argument that modification of Janoff to include PEG chains would alter the ability of the ligand to competitively bind and reduce toxicity; and (3) that Popescu teaches treatment of Listeria, as does the present specification, and, therefore, since the bacteria are the same the sites of infection are the same.

2. The Applicants' Rebuttal

A. Combination of Janoff with Yoshioka

The Examiner characterizes the teaching of Janoff as follows:

"Janoff teaches gentamycin containing liposomes (note the abstract, examples and claims). Janoff however, does not teach that phospholipids are used in the formation of liposomes, be attached with the hydrophilic polymer such as polyethylene glycol (PEG)" (Office action dated July 9, 1999, page 7).

It is the applicants position that to characterize the teaching of Janoff in this way ignores the teaching of Janoff as a whole. A more correct characterization of Janoff is to summarize that Janoff is concerned with reducing the toxicity of drugs (Col. 6, lines 54-

56). The drugs for use in Janoff's invention include drugs having toxic side effects mediated by binding to endogenous cellular substrates, typically lipids (Col. 6, lines 59-63). To achieve a reduced drug toxicity, Janoff administers the drug in the presence of a lipid "ligand" capable of binding to the drug toxicity receptor to prevent interaction between the drug and its receptor (Col. 7, lines 35-44). Thus, the head group of the lipid must be capable of binding to the cellular receptor for the drug (Col. 10, lines 2-4). Alternatively, the phosphate moieties on the lipid head group form a complex with the drug to inhibit binding of the drug to the receptor (Col. 11, lines 30-42; Col. 9, line 52-Col. 10, line 2).

In considering the teaching of the Janoff reference as a whole, it is clear that the lipid ligand, and in particular the head group of the lipid, must be freely available to bind to a cellular toxicity receptor or to bind with the drug for complex formation.

It is well recognized in the art that conjugation of a PEG chain to a lipid head group forms a stearic "shell" about the lipid head group. Such a shell is depicted in Figs. 3A-3B of the enclosed Hristove *et al.* reference. Intuitively, such a shell must hinder binding interaction between the lipid head group and a receptor. A variety of studies, such as the one discussed in the enclosed paper of Noppl-Simson and Needham[3], where PEG is reported to interfere with binding interactions.

In light of this, there is simply no motivation to modify Janoff to include a PEG chain as taught by Yoshioka.

B. Combination of Popescu with Yoshioka

The Examiner argues that Popescu teaches liposomes containing gentamycin. The Examiner combines the teaching of Popescu with Yoshioka on the grounds that it would be desirable to modify the liposomes of Popescu with PEG chains as taught by Yoshioka to prevent adsorption of plasma proteins on the liposomes.

The applicants assert that [4]the Examiner is failing to consider the teaching of Popescu as a whole, and is improperly picking and choosing portions of the reference to support a position.

Popescu is more fairly characterized as a teaching of a

[3] Noppl-Simson and Needham, *Biophys. J*, 70(3):1391 (1996).

liposomal composition for treating infections which reside in the reticuloendothelial system (RES), specifically in macrophages (Col. 4, lines 49-64). As such, the composition described by Popescu is intended to be, after *in vivo* administration, taken up by phagocytic cells of the RES (Col. 5, lines 2-10).

The Examiner appears to ignore this purpose of Popescu, asserting that on Columns 4 and 5 Popescu only refers to the sites the bacteria is likely to infect. Applicants point the Examiner specifically to Col 4., lines 49 which reads:

> In one scheme, SPLVs are used to deliver therapeutic agents to sites of intracellular infections. Certain diseases involve an infection of cells of the reticuloendothelial system, *e.g., brucellosis.* These intracellular infections are difficult to cure for a number of reasons (1) because the infectious organisms reside within the cells of the reticuloendothelial sustem, they are sequestered from circulating therapeutic agents which cannot cross the cell membrane..."

> On Col. 4, beginning at line 65:
> "According to one mode of the present invention, SPLVs containing an appropriate biologically active compound are administered (preferably intraperitoneally or intravenously) to the host organism or potential host organism... *Since phagocytic cells internalize SPLVs*, the administration of an SPLV-encapsulated substance that is biologically active against the infecting agent organism will result in directing the bioactive substance to the site of infection (*emphasis added*). "

In contrast, the liposomes of the present invention are intended to avoid uptake by the RES, in order to concentrate the liposomes at a site of tissue infection. Modification of Popescu's formulation, as suggested by the Examiner, to include PEG-derivatized phospholipids of Yoshioka, would defeat its ability to be taken up by the RES, thereby defeating the intended purpose of the composition.

Accordingly, applicants respectfully request withdrawal of the rejection under 35 U.S.C. §103.

VI. Conclusion

In view of the above remarks, the applicants submit that the claims now pending are in condition for allowance. A Notice of Allowance is, therefore, respectfully requested.

If in the opinion of the Examiner a telephone conference would expedite the prosecution of the subject application, the Examiner is encouraged to call the undersigned at (650) 324-0880.

Respectfully submitted,

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Date: 10/21/99

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